NON-ADDITIVE CULTURES FOR ALGAE

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Phycological literature is replete with a variety of techniques for the handling of algae once they have been collected. Aside from the rather obvious requirement for examination of the living material as expeditiously as possible, one might then choose from a variety of methods such as are found in Smith (1950), Pringsheim (1946a, 1946b), or Prescott (1954). The goal of the investigator certainly suggests the proper method or methods to be used. During several years of collecting fresh-water algae from a variety of localities in New England, this author has utilized a method that is much less sophisticated than those usually employed, yet as a result, has been able to achieve a greater than usual use of the material. It is the purpose of the author to present a brief discussion of the technique and the use that may be made of it.

The collections by the author, principally in the towns of Medfield, Walpole, and Sharon, all within the area of Norfolk County, Massachusetts, have been attempts to isolate causes of and mechanisms for algal periodicity. Standard techniques of collection have been utilized, and whenever possible or practical, at least two methods of preservation of representative specimens. More often than not, considerable quantities of material were left after initial use and it is this extra material which has been the source of much interest and work.

The method employed is simplicity in itself. The wide-mouthed jar with the remaining material was tightly capped with a Bakelite cap, and placed on a window shelf with a southwesterly exposure. The window is shaded by a rather dense stand of Eastern White Pine (*Pinus Strobus* L.) and gets direct sunlight only during the latter part of the afternoon. The material in the jars is not monitored critically on an exacting or rigid schedule, rather, casual observations are used to check on the material within the jars, with

detailed examination given to those that exhibit distinct changes in the material.

Gilbert Smith (1950), in a discussion of the techniques and problems of culturing algae makes several points that seem appropriate to mention here. He suggests that "there is no general method by which collections of algae may be kept alive in the laboratory for indefinite periods"; and that "algae maintained alive under laboratory conditions so frequently develop abnormal thalli that one should compare the laboratory-grown algae with freshly gathered specimens to avoid misleading conclusions." Finally he makes the point that, because the new environment favors the growth of only certain of the algae within the culture, changes in the structure of the association, are to be expected particularly in regard to the relative numbers of each species that are present.

It is the opinion of this author that Smith's initial premise is not valid for all conditions of culture. He does suggest that water from the original habitat is often the best culture solution, a fact that this investigator has also found to be the case. However, based on the experience with cultures in the author's own laboratory it is entirely possible to hold algal cultures in a fresh, useful condition for more than a year. It might indeed be possible to hold them for longer periods than that. A number of jars now on the shelves have been there for that period and as of now show no signs of deterioration.

Similarly the investigations leading to this paper do not entirely support the premise that abnormalities are to be commonly encountered. Obviously the particular species, or even the genera used, may have a considerable bearing on this point. The only material that this author found dealing with algal abnormalities (Cushman, 1904) dealt with abnormalities that occur during division and following ingestion by crustaceans. As species were encountered in the cultures under discussion careful measurement against published standards (e.g. Prescott, 1962), suggests that abnor-

malities are extremely rare. Only one instance was found in this case, and it was deemed to be of no significance, as will be shown later.

Smith's third premise, that the numbers of each species change because of the changed environment, is held to be entirely valid, and as a matter of fact, is one of the most desirable attributes to this method of culture. As the conditions within the jar change, the response by the algae yields quite a different association, often involving the loss of one or more of the original forms, and the emerging of others as dominants. The most obvious variable in the present case is that of temperature. The original habitats having not only greater extremes, but due to changing environmental conditions consistent with seasonal change, quite different averages. The two different cultures used as illustrations in this paper, for example, have had temperatures with extremes of 15° C, to 32° C, although the general average has run between 20-24° C, as might be expected within a house under normal conditions of living. The close proximity to the window glass accounts for the greater extremes.

One of the sets of material has been "on the shelf" since April of 1969. Two jars, quite different in original collection material, comprise one set. Both jars were collected from the same stream pool, at the same time, under the same conditions. One jar (Jar A hereafter) held *Tetraspora lubrica* (Roth) C. C. Agardh, with a small amount of dead grass stems, and a like amount of *Fontinalis* (*Fontinalis gigantea* Sulliv.), a rather common aquatic moss in this region. As might be expected the abnormally warmer environment led to the complete destruction of the *Tetraspora*, a species that is usually a cold-water type. The second jar, collected from a point that was slightly more in midstream than the former jar, contained a quantity of *Fontinalis*, to which was attached a small amount of *Tetraspora*, and a considerable quantity of *Microspora amoena* (Kuetz.) Rabenhorst. This second jar is Jar B, for the purposes of this discussion.

Casual examination of both jars in late February of 1970 revealed a considerable change. Jar A now showed as a dominant form *Nitella*, which close examination showed to be *N. flexilis* (L.) C. A. Agardh. Jar B also had *Nitella* as a prominent species, but an even greater growth of *Vaucheria geminata* (Vauch.) De Candolle. The *Nitella* in each jar was not found in the initial examination of the material in 1969, hence it must be assumed that the original collection included reproductive bodies in some form. The *Vaucheria* might easily have been missed among the *Fontinalis*, if as is suspected, it existed in rather minute amounts in the original material.

Jar A contained no other filamentous or multicellular alagae, but the surface of the *Nitella* was crowded with epiphytic species. The four most numerous species being *Characiopsis longipes* (Rabenh.) Brozi, *Characium pringsheimii* A. Braun, *C. ornithocephalum* A. Braun, and *C. rostratum* Reinhard. The most common species represented in the detritus on the bottom of the jar was *Pleurotaenium ehrenbergii* (Breb.) De Bary. This species was not found in the living state, however, the identification was made from semi-cells in the litter.

Jar B contained an abundance of the filaments of *Hyalotheca dissiliens* (Smith) De Brebisson, and one species of *Oedogonium* which had not developed the reproductive structures requisite for precise identification. In addition, several other desmids were common: *Closterium leibleinii* Kuetzing being the most abundant, while *C. ehrenbergii* Meneghini and *Euastrum affine* Ralfs were encountered less frequently.

Earlier mention was made of one species that did not conform to the published size ranges for it. This species was *Microspora amoena* (Kuetz.) Rabenhorst, and it showed to be larger than normal by some 3 microns in both length and diameter. It otherwise met all the required taxonomic characteristics and was assigned by this investigator to that species, especially as it had been identified in the original collection, and at that time no characters were

unusual. This seems to be the sort of situation described by Smith, but whether or not it is as widespread as he seemed to think is open to question.

Neither of the jars examined in 1970 showed many motile algae, and this circumstance agrees with Smith, his suggestion being that this type went first in culture and should be sought for first in examination of material. Two species were found infrequently in Jar B. *Eudorina elegans* Ehrenberg and *Synura uvella* Ehrenberg were among the strands of material in that jar.

While the previous example consisted of members of the Chlorophyta, the presence of species of Cyanophyta, for example, can be shown as easily. A collection from a small, slow, feeder stream in the same general locality, cultured from March of 1969 and lately examined, revealed a considerable quantity of *Hapalosiphon hibernicus* West & West, both as part of an association forming a mat on the surface of the water in the jar, and entangled down in the jar among the other filaments. The other member of the surface mat was a species of *Anabaena* which lacked sufficient taxonomic characters to identify the species.

This culture was and is well-filled, it being impossible to look clear through the jar. A large part of this dense mass was composed of *Fontinalis*, and the remainder partly filamentous Chlorophyta and partly detritus. The experience of this investigator has been that the genus *Microspora* is one of the most abundantly represented in most collections, at least within the Neponset River watershed. In addition to the filamentous desmid *Hyalotheca dissiliens*, three species of *Microspora*, *M. quadrata* Hazen, *M. stagnorum* (Kuetz.) Lagerheim, and *M. pachyderma* (Wille) Lagerheim made up the bulk of the filamentous material.

One last example to show the versatility of the method can be taken from a culture collected from a small, but deep and swiftly moving stream area considerably upstream from the other sites. The original material was collected from twigs and other vegetative debris hanging in the stream. The original material was primarily *Tetraspora lubrica*, but found among the specimens was *Rhizoclonium*

hieroglyphicum (Ag.) Kuetzing, *Pandorina morum* (Mueller) Bory, three clearly separate species of *Spirogyra*, one species of *Mougeotia*, and several strands of *Microspora amoena*.

Unlike the earlier examples, the long period prior to re-examination was not required in this instance. Two months after the "on the shelf" routine started, this investigator was able to isolate *Spirogyra denticulata* Transeau as one of three previously unidentified types. By contrast, one of the other species of *Spirogyra* had all but vanished.

On the basis of his experience with this rather simple method of algal culture this investigator is very much satisfied with the results that can be obtained. Not only can a much more representative grouping of the algae from a given station be obtained with patience and care, but very little time is required for maintenance.

One other advantage can be realized by the investigator who has the opportunity to work with students at almost any level. These mixed cultures provide an interesting challenge for the student and the teacher together, and as this writer has found, often open the channels of communication much more quickly than the uni-algal, more stereotyped material that is frequently encountered. Then too it frees the instructor from the problems of institutional orders and commercial suppliers, no small matter in this day of crowded schedules and classrooms.

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LITERATURE CITED

CUSHMAN, J. A. 1904. Division in desmids under pathological conditions. Rhodora **6(72)**: 234.

PRESCOTT, G. W. 1954. *How to Know The Fresh Water Algae.* William C. Brown Co. Dubuque.

———————. 1962. *Algae of the Western Great Lakes Area.* William C. Brown Co. Dubuque.

PRINGSHEIM, E. G. 1946a. *Pure Cultures of Algae, Their Preparation and Maintenance.* Cambridge.

———————. 1946b. The biphasic or soil-water culture method for growing algae and Flagellata. Jour. Ecol. **33**: 193-204.

SMITH, G. M. 1950. *Fresh-Water Algae of the United States.* 2nd. Ed. McGraw-Hill Book Co. N.Y.